

Application No. 09/754,014
Amendment Dated January 28, 2005
Revision of Reply filed 08/18/04 to Office Action of 4/20/04

Amended Listing of Claims:

Claims 1 – 4 (cancelled)

Claim 5 (currently amended): A plasmid for expression of recombinant ~~eucaryotic~~ eukaryotic genes comprising:

a first transcription unit comprising a first transcriptional control sequence transcriptionally linked with a first 5'-untranslated region comprising a first synthetic intron, a first coding sequence, and a first 3'-untranslated region/poly(A) signal, wherein said first synthetic intron is between said first transcriptional control sequence and said first coding sequence; and

a second transcription unit comprising a second transcriptional control sequence transcriptionally linked with a second 5'-untranslated region comprising a second synthetic intron, a second coding sequence, and a second 3'-untranslated region/poly(A) signal, wherein said second synthetic intron is between said second transcriptional control sequence and said second coding sequence,

wherein the first and second synthetic introns both comprise 5' splice sites having a sequence of SEQ ID NO:10 residues #1 through #9, and/or branch points having a sequence of SEQ ID NO:10 residues #16 through #22, and/or 3' splice sites having a sequence of SEQ ID NO:10 residues #25 through #45.

Claims 6 – 9 (cancelled).

Claim 10 (currently amended): A plasmid for expression of recombinant ~~eucaryotic~~ eukaryotic genes, comprising an intron having variable splicing, a first coding sequence, and a second coding sequence, wherein the plasmid comprises:

a transcriptional control sequence transcriptionally linked with a first coding sequence and a second coding sequence;

a 5'-untranslated region;

an intron 5' to said first coding sequence;

an alternative 3' splice site located between the first and second coding sequence; and

a 3'-untranslated region/poly(A) signal,

wherein the intron comprises a 5' splice site having a sequence of SEQ ID NO:10 residues #1 through #9, and/or branch points having a sequence of SEQ ID NO:10 residues #16 through #22, and/or 3' splice sites having a sequence of SEQ ID NO:10 residues #25 through #45.

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Claims 11 - 13 (cancelled).

Claim 14 (currently amended): A plasmid for expression of recombinant ~~eucaryotic~~ eukaryotic genes comprising:

- a transcriptional control sequence transcriptionally linked with a first coding sequence, an IRES sequence, a second coding sequence, and a 3'-untranslated region/poly(A) signal, wherein said IRES sequence is between said first coding sequence and said second coding sequence; and
- a synthetic intron between said transcriptional control sequence and said first coding sequence, wherein the synthetic intron comprises a 5' splice site having a sequence of SEQ ID NO:10 residues #1 through #9, and/or branch points having a sequence of SEQ ID NO:10 residues #16 through #22, and/or 3' splice sites having a sequence of SEQ ID NO:10 residues #25 through #45.

Claims 15 - 49 (cancelled).

Claim 50 (previously presented): The plasmid of claim 5 wherein the first and second synthetic introns both comprise 5' splice sites having the sequence of SEQ ID NO:10 residues #1 through #9, branch points having a sequence of SEQ ID NO:10 residues #16 through #22, and 3' splice sites having a sequence of SEQ ID NO:10 residues #25 through #45.

Claim 51 (currently amended): The plasmid of claim 10 wherein the ~~first and second synthetic introns both comprise~~ intron comprises a 5' splice sites having the sequence of SEQ ID NO:10 residues #1 through #9, a branch points point having a sequence of SEQ ID NO:10 residues #16 through #22, and both the 3' splice sites having site and the alternative 3' splice site have a sequence of SEQ ID NO:10 residues #25 through #45.

Claim 52 (previously presented): The plasmid of claim 10 wherein either the 3' splice site or the alternative 3' splice site is weakened with respect to the other.

Claim 53 (previously presented): The plasmid of claim 10 wherein either the 3' splice site or the alternative 3' splice site is weakened with respect to the other by changing three consecutive T's to A's.

Claim 54 (previously presented): The plasmid of claim 10 wherein the 3' splice site has a sequence of SEQ ID NO:10 residues #25 through #45 with residues #32 through #34 replaced by AAA and the alternative 3' splice site has a sequence of SEQ ID NO:10 residues #25 through #45.

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Claim 55 (currently amended): The plasmid of claim 14 wherein the synthetic intron comprises the 5' splice site having the sequence of SEQ ID NO:10 residues #1 through #9, ~~a branch points-point~~ having a sequence of SEQ ID NO:10 residues #16 through #22, and ~~a 3' splice sites site~~ having a sequence of SEQ ID NO:10 residues #25 through #45.

Claims 56 - 64 (cancelled).

Claim 65 (new): A synthetic transcription unit for efficient and accurate expression of recombinant eukaryotic genes, the transcription unit comprising a synthetic intron comprising:
a 5' splice site having a sequence MAGGTRAGT, wherein M = C or A and R = G or A (SEQ ID NO:14); a branch point having a sequence YNYTRAY, wherein Y = C or T, R = A or G and N = any base (SEQ ID NO: 16); and a 3' splice site having a sequence Y₁₆NYAGG, wherein Y = C or T, and N = any base (SEQ ID NO: 18).

Claim 66 (new): The synthetic transcription unit of claim 65, wherein the sequence of the 3' splice site is adjusted to contain 7 consecutive T residues.

Claim 67 (new): The synthetic transcription unit of claim 65, wherein the 3' splice site has a sequence TTCTTTTTTCTCTTCNYAGG, wherein Y = C or T, and N = any base (SEQ ID NO:19).

Claim 68 (new): The synthetic transcription unit of claim 65, wherein the branch point has a sequence TACTAAC (SEQ ID NO:17).

Claim 69 (new): The synthetic transcription unit of claim 65, wherein the branch point and the 3' splice site together have a sequence of TACTAACGGTCTTTTTTTCTCTTCACAGG (SEQ ID NO:13, residues #93 through #122).

Claim 70 (new): The synthetic transcription unit of claim 65, wherein an actual branch point in the branch point is located 24 to 38 nucleotides upstream from a site of splicing in the 3' splice site.

Claim 71 (new): The synthetic transcription unit of claim 65, wherein the synthetic intron is from 90 to 200 nucleotides in length.

Claim 72 (new): The synthetic transcription unit of claim 65, wherein the synthetic intron has a sequence CAGGTAAGTGTCTTC-N₇₇-TACTAACGGTCTTTTTTTCTCTTCACAGG (SEQ ID NO:13).

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Claim 73 (new): A synthetic transcription unit for efficient and accurate expression of recombinant eukaryotic genes, the transcription unit comprising a synthetic intron of from about 90 to about 200 nucleotides in length which has a 5' splice sequence of CAGGTAAGT (SEQ ID NO:13, residues #1 through #9), a branch point sequence of TACTAAC (SEQ ID NO:13, residues #16 through #22), and a 3' splice site having a sequence Y₁₆NYAGG, wherein Y = C or T, and N = any base (SEQ ID NO: 18).

Claim 74 (new): The synthetic transcription unit of claim 73, wherein the 3' splice site has the sequence TTCTTTTTTCTCTTCACAGG (SEQ ID NO: 13 residues # 102 through #122).

Claim 75 (new): The synthetic transcription unit of claim 73, wherein an actual branch point in the branch point is located 24 to 38 nucleotides upstream from a site of splicing in the 3' splice site.

Claim 76 (new): The synthetic transcription unit of claim 73, wherein the synthetic intron has a sequence CAGGTAAGTGTCTTC-N₇₇-TACTAACGGTTCTTTTTTCTCTTCACAGG (SEQ ID NO:13).